AFRL-HE-WP-TR-2005-0012

United States Air Force Research Laboratory

Determination of Partition Coefficients for a Mixture of Volatile Organic Compounds in Rats and Humans at Different Life Stages

Deirdre A. Mahle
Jeffery M. Gearhart
Richard J. Godfrey

Alion Science and Technology
P.O. Box 31009
Dayton, OH 45437-0009

David R. Mattie
Robert S. Cook
Claude C. Grigsby

Air Force Research Laboratory
Human Effectiveness Directorate
Applied Biotechnology Branch
Wright-Patterson AFB, OH 45433-5707

May 2004

FINAL REPORT FOR THE PERIOD SEPTEMBER 2002 TO APRIL 2004

20050805 018

Air Force Research Laboratory
Human Effectiveness Directorate
Biosciences and Protection Division
Applied Biotechnology Branch
Wright-Patterson AFB, OH 45433-5707

NOTICES

DISCLAIMER

TECHNICAL REVIEW AND APPROVAL

AFRL-HE-WP-TR-2005-0012

The animal use described in this study was conducted in accordance with the principles stated in the "Guide for the Care and Use of Laboratory Animals", National Research Council, 1996, and the Animal Welfare Act of 1966, as amended.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE DIRECTOR

//SIGNED//

MARK M. HOFFMAN
Deputy Chief, Biosciences and Protection Division
Air Force Research Laboratory

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*
February 2005

2. REPORT TYPE
Final Tech Report

3. DATES COVERED *(From - To)*
September 2002-April 2004

4. TITLE AND SUBTITLE
Determination of Partition Coefficients for a Mixture of Volatile Organic Compounds in Rats and Humans at Different Life Stages

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
62202F

5d. PROJECT NUMBER
1710

5e. TASK NUMBER
1710D4

5f. WORK UNIT NUMBER
1710D432

6. AUTHOR(S)
*Mahle, Deirdre A.; Gearhart, Jeffery M.; Godfrey, Richard J.
Mattie, David R.; Cook, Robert S.; Grigsby, Claude C.(AFRL/HEPB, Wright-Patterson AFB, OH)

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
ManTech Environmental Technology, Inc.
P.O. Box 31009
Dayton, OH 45437-0009

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Air Force Research Laboratory
Human Effectiveness Directorate
Biosciences and Protection Division
Applied BioTechnology Branch
Wright-Patterson AFB, OH 45433-5707

10. SPONSOR/MONITOR'S ACRONYM(S)
AFRL/HEPB

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
AFRL-HE-WP-TR-2005-0012

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Pharmacokinetic differences in child, adult and elderly populations remain ill-defined. Partition coefficients (PCs) are an integral component of pharmacokinetic models and determining differences in tissue partitioning of volatile organic chemicals across life stages can help reduce the uncertainty in risk assessment. PCs for methylene chloride, methyl ethyl ketone, chloroform, benzene, trichloroethylene and perchloroethylene were determined in blood, liver, kidney, fat, muscle and brain of male adult (60 days) and aged (22 months) Sprague-Dawley (S-D) rats (n = 10) and male and female S-D pups (n = 10 litters) at postnatal day 10, and in human blood from male and female pediatric and adult donors. Human adult and pediatric blood:air PCs were similar regardless of sex; values for the solvents ranged from 8.3 ± 2.3 (adult female, benzene) to 191.3 ± 5.7 (pediatric male, methyl ethyl ketone). Liver:air methylene chloride PCs for adult male rats averaged 20.4 ± 4.8 compared to 12.7 ± 1.0 for male rat pups.

15. SUBJECT TERMS
blood liver kidney muscle brain

16. SECURITY CLASSIFICATION OF: a. REPORT	b. ABSTRACT	c. THIS PAGE	17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON / 19b. TELEPHONE NUMBER *(Include area code)*
Unclassified	Unclassified	Unclassified	SAR	38	Deirdre A. Mahle (AFRL/HEPB) / 937-904-9496

THIS PAGE INTENTIONALLY LEFT BLANK

PREFACE

This non-peer-reviewed report details the work performed to determine the PCs for selected halogenated organic compounds in blood and tissues of young, adult and elderly rats and in human blood. This research began on 1 September 2002 and was completed on 30 April 2004 under Department of the Air Force Contract No F33615-00-C-6060. Dr. David R. Mattie served as the Contract Technical Monitor for the U.S. Air Force, Air Force Research Laboratory, Applied Biotechnology Branch (AFRL/HEPB) and Dr. Darol Dodd served as Program Manager for the ManTech/GEO-CENTERS Joint Venture Contract (F33615-00-C-6060). Work was also performed by Air Force personnel under work unit 1710D432. This work was sponsored by the U.S. Environmental Protection Agency under Interagency Agreement DW-57-93960601-0 with Dr. John Lipscomb, USEPA, ORD/NCEA, Cincinnati, OH, serving as the U.S. Environmental Protection Agency project monitor.

During the experimental portion of this project, the primary author was employed as a government contractor with ManTech Environmental Technology, Inc., Dayton, OH. Deirdre Mahle is currently a government civilian with AFRL/HEPB.

Special acknowledgements and appreciation are extended to Ms. Lisa Ludeman, Pediatrics Laboratory, Pediatric Clinic, and Mr. Dan Fisher, Hematology Section, Clinical Laboratory Services, both of the 74th Medical Group, Wright Patterson AFB, OH, for obtaining and providing pediatric and adult blood samples for PC determination.

Appreciation and acknowledgements are also given to Teresa R. Sterner of Operational Technologies Corporation, Beavercreek, OH, for technical assistance in the preparation of this report.

All studies involving animals were conducted under a program of animal care accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International, and in accordance with the "Guide for the Care and Use of Laboratory Animals", National Research Council (1996).

The Human Subject Testing Committee, Air Force Research Laboratory and Institutional Review Board, 74th Medical Group, Wright-Patterson AFB, OH approved the human subject testing protocol, "Development of Pharmacokinetic Parameters in Human Tissues and Blood" (FWR 2003-0010-E and FWP20030010E, respectively), as an exempt protocol in accordance with Section 219.101 par (b)(2) of 32 CFR 210.

THIS PAGE INTENTIONALLY LEFT BLANK

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

Table .... Page

Figure .... Page

ABBREVIATIONS

ATSDR	Agency for Toxic Substances and Disease Registry
BEN	Benzene
CFM	Chloroform
CO_2	Carbon Dioxide
DCM	Dichloromethane
GC	Gas Chromatography
MEK	Methyl Ethyl Ketone
PBPK	Physiologically Based Pharmacokinetic
PC	Partition Coefficient
PCE	Perchloroethylene
PND	Postnatal Day
s.d.	Standard Deviation
S-D	Sprague-Dawley
TCE	Trichloroethylene
USEPA	United States Environmental Protection Agency
VOCs	Volatile Organic Compounds

THIS PAGE INTENTIONALLY LEFT BLANK

DETERMINATION OF PARTITION COEFFICIENTS FOR A MIXTURE OF VOLATILE ORGANIC COMPOUNDS IN RATS AND HUMANS AT DIFFERENT LIFE STAGES

INTRODUCTION

Physiologically based pharmacokinetic (PBPK) models are valuable tools used to describe the absorption, distribution, metabolism and elimination of xenobiotics and to assess the risk of exposure to a chemical based on scientific data. An integral parameter required to develop a PBPK model is the measure of a chemical's solubility or partitioning in a tissue versus the blood or plasma volume (Ramsey and Andersen, 1984). The partition coefficient (PC) is a ratio of the concentrations achieved between two different media at equilibrium, such as blood:air or blood:tissue. Traditionally, PBPK models have been developed for adult rodents to be extrapolated to humans. However, differences in blood and tissue composition at varying stages of life may affect the measured PC. Infants and children have lower levels of serum protein and lipid (Avery, 1981), and it has been shown that age affects the blood:gas PC for volatile anesthetics (Lerman *et al.*, 1984). Utilizing the same blood:tissue PCs for children and adults could result in significant discrepancies between model predictions of target tissue dose and actual tissue concentrations.

Because of historic or current industrial and military usage, volatile organic compounds (VOCs) are of interest to regulatory agencies such as the U.S. Environmental Protection Agency (USEPA) and the Agency for Toxic Substances and Disease Registry (ATSDR). Groundwater sources are commonly contaminated with mixtures of VOCs, and cleanup is difficult and costly. Occupationally, the risk of dermal and inhalation exposure to VOCs is high. In addition, contaminated drinking water poses an oral exposure route for residential risk. As nonpolar and lipophilic compounds, VOCs transport easily across lipid membranes and are retained in fat. The VOCs of interest for this research are methyl ethyl ketone (MEK), methylene chloride (DCM), trichloroethylene (TCE), perchloroethylene (PCE), chloroform (CFM) and benzene (BEN). These solvents are of concern to both the Department of Defense and the USEPA in terms of environmental and human health risks. Currently, many risk assessments use default

uncertainty factors when science-based values are not available. In order to replace these default uncertainty factors with science-based chemical specific data and to reduce variance between humans and animals as well as among humans, it is necessary to develop biologically based information and underlying biochemical mechanisms.

Limited scientific information and data are available on how volatile organic solvents distribute in blood and tissues of children, adults and the elderly. This information can be very useful for improving the estimation of the health risk associated with exposure to organic solvents. The main questions that need to be answered are: 1) Are there differences in tissue solubility of VOCs in children, adults and the elderly? 2) What are the differences in blood composition of children, adults and the elderly that may affect tissue partitioning of volatile organic chemicals? 3) Can computer models be developed to describe the chemical dose to tissues and organs across rodent and human populations at varying life stages?

The objective of this research is to determine tissue and blood partitioning coefficients of volatile organic chemicals in pediatric, adult and elderly rodents, and in blood of pediatric and adult humans. Also, blood components of protein, cholesterol and triglyceride which may affect tissue partitioning were quantitated. The data collected from this research will be used to develop and refine physiologically based pharmacokinetic models to describe tissue dosimetry across rodent and human populations at varying life stages.

MATERIALS AND METHODS

Chemicals

Six test chemicals were purchased. Source and percent purity for each of these chemicals are shown in Table 1.

Table 1. Source and purity of study chemicals used to determine partition coefficients

Chemical	Source	Verified Purity (%)
Methylene chloride (DCM)	Sigma-Aldrich	99.8
Methyl ethyl ketone (MEK)	Sigma-Aldrich	99.8
Chloroform (CFM)	Fisher Biotech	100
Benzene (BEN)	Sigma-Aldrich	100
Trichloroethylene (TCE)	Sigma-Aldrich	100
Perchloroethylene (PCE)	Sigma-Aldrich	99.6

The scope of this work required the determination of PCs for multiple chemicals. Previous work conducted in this laboratory (Fisher *et al.*, 1997) showed that PCs can be determined using a limited mixture of volatile organic solvents. The present research used a modified method of analysis based on the work of Fisher *et al.* (1997). By using a mixture of volatile solvents, the number of animals required was greatly diminished. The volumes of each chemical added to a 10 L Tedlar gasbag containing 5 L of ambient air to obtain individual concentrations of 10,000 ppm are shown in Table 2.

Table 2. Volume added to Tedlar bag containing 5 L of ambient air to obtain 10,000 ppm concentration for each chemical

Chemical	Volume (μL)
Methylene chloride	134
Methyl ethyl ketone	187
Chloroform	168
Benzene	185
Trichloroethylene	187
Perchloroethylene	213

Tissue and Blood Collection

Sprague-Dawley (S-D) rats were purchased from Charles-Rivers Laboratory (Raleigh, NC). Adult male rats were approximately 60 - 70 days old upon arrival at the vivarium facility and were used immediately. Aged male rats were 12 months old upon arrival and were housed

until they reached 22 months of age. Timed pregnant female rats arrived at gestation day 8-10 and were housed with their litters after parturition until postnatal day (PND) 10. For this study parturition was designated as PND 1. No animals were exposed *in vivo*.

All animals (adult male, aged male, PND 10 dams and PND 10 pups) were euthanized by carbon dioxide (CO_2) inhalation for not less than 5 minutes. Blood was drawn from the inferior vena cava, and liver, kidney, fat, muscle and brain were then removed for PC determination. Each adult rat provided sufficient quantity of tissue and blood to give an "n" of 1 per rat (total n = 10). PND 10 litters were separated by sex, and blood and tissues were pooled by sex within each litter to provide an n of 1 for each litter (total n = 10).

In order to optimize the time to reach equilibrium for the selected chemicals and tissues, 12 additional adult male rats were euthanized by CO_2 inhalation, and blood, liver, kidney, fat, muscle and brain removed. Four time points, ranging from 2 to 4 hours were tested (n = 3/time point) to determine the optimal time of incubation prior to analysis. This blood and tissue were also used for refinement of the analytical method and for standard calibration.

Human blood was supplied by the Wright-Patterson Air Force Base Medical Center from pediatric patients ranging from 3-10 years old and adult patients ranging from 20-82 years. Blood was collected by venipuncture in EDTA collection tubes and stored at 4°C. The samples were received within 24 hours of collection and stored at 2-8°C until use. Human blood was analyzed within approximately 48 hours.

Serum or plasma protein, cholesterol and triglyceride levels were determined in all blood samples, human and rat, using an IDEXX Vet Test® clinical chemistry analyzer (Model VT8008, IDEXX Laboratories, Westbrook, ME). The PND 10 pups from the pediatric PC determination phase did not generate sufficient blood volume to complete the lipid and protein assays; therefore, a separate set of PND 10 rat pups was used for clinical chemistry determinations. Adult rats supplied 7 to 9 mL of blood, enough for both partition determination and lipid and protein content assays.

Partition Coefficient Determination

The vial equilibration method of Gargas *et al.* (1989) was used to determine PCs. The blood and tissues were incubated with the selected chemical mixture until equilibrium was achieved between the headspace and tissue. Headspace samples were automatically removed and analyzed by gas chromatography. Headspace concentrations of each compound were analyzed by a Hewlett-Packard Gas Chromatograph (GC) (Model 5890, Agilent Technologies, Inc., Palo Alto, CA) equipped with a 0.53 mm x 30 m volatile organic column and a flame ionization detector. The carrier gas was helium at 4 mL/min He carrier with make-up flow at 26 mL/min. The inlet temperature was 120°C, and detector temperature was 150°C. The temperature program was as follows: 30°C for 3 min; 2°C/min to 54°C; 7°C/min to 110°C for 3 min for a total time of 26 min.

The Tekmar Headspace Sampler (Model 14-4401-000, Teledyne Technologies, Inc., Los Angeles, CA) parameters were as follows: Platen - 37°C; PlatenEquil - 0.10; SamplEquil - 210.0; Vial Size - 22 mL; Tekmar Cryofocusing Module (Model 14-2530-000) - 3 min at -15°C; 1 mL Sample Cap Inject 0.4 min at 120°C; Valve - 110°C; Line - 110°C; Capillary Union Heater - 110°C; GC Cycle Time 33 min.

Quality Control

Tissue blanks were run to ensure contamination was not present. Standard curves were determined initially and periodically for each individual chemical and compared with standard curves obtained from each chemical in the mixture.

Headspace vials (20 mL) using Teflon coated septa containing 0.5 mL from a 10,000 ppm Tedlar bag were tested over a 16 hr period (average length of time to analyze all of the samples in the autosampler). No statistical decrease in concentration was noted. Tedlar bag stability was evaluated and determined to have a stability of not greater than one calendar week.

Sample volumes of 5, 25, 100, 250, 500 and 1000 µL from a 10,000 ppm Tedlar bag (above) were tested in duplicate using the previously established method. All standard curve slopes obtained were linear, with R values > 0.993.

Statistical Analysis

Non-parametric tests were used for all comparisons of rat data. Two separate analyses were performed. The first analysis used the Wilcoxon Signed Rank test to compare female and male pups (paired by litter). The second analysis used the Kruskal-Wallis One-Way Layout test for an overall comparison of male pups, adult males and aged male with paired comparisons using the Wilcoxon Rank Sum test. All paired tests were 2-tailed and had an error level of 0.05.

For human data, two-tailed two-sample t-tests were performed to determine the significance of differences between female and male pediatrics, female and male adults, and between pediatrics and adults for each gender. Analyses of variance were performed to test for main effects of age group (pediatric vs. adult), gender and the age group-gender interaction. All paired tests had a per comparison error level of 0.05.

RESULTS

Rat Partition Coefficients

Table 3 lists the tissue:air PC values for blood, liver, kidney, fat, muscle and brain in rats ranging from PND 10 to 22 months for the 6 chemicals in this study. Compiled data are listed in Appendix A. Aged rats had consistently higher PCs in every tissue for methylene chloride, chloroform, benzene, trichloroethylene and perchloroethylene, with the exception of muscle, when compared to adult rats and PND 10 pups. PND 10 pup blood and brain had higher measured PCs for methyl ethyl ketone as compared to adult and aged rats. For every chemical tested, muscle PCs were higher in male and female PND 10 pups, as compared to the adult and aged rat groups.

Table 4 lists the levels of protein, cholesterol and triglycerides in rat serum. Aged rats had an approximately 50% increase in serum triglyceride level as compared to PND 10 and adult rats, which may correspond to the increase in PC values. Cholesterol levels, however, dropped about 31% from PND 10 to 22 months. Total protein levels in adult and aged rats were roughly twice the protein levels in 10 day old pups.

Table 3. Rat partition coefficient values of blood, liver, kidney, fat, muscle and brain for methylene chloride, methyl ethyl ketone, chloroform, benzene, trichloroethylene and perchloroethylene (mean ± s.d.)

DCM	Blood	Liver	Kidney	Fat	Muscle	Brain
PND10 male (*n=10*)	14.4 ± 0.7	13.0 ± 0.9	9.9 ± 0.4	86.3 ± 19.7	16.2 ± 3.6	9.0 ± 0.5
PND10 female (*n=10*)	14.4 ± 0.7	12.3 ± 0.8	10.0 ± 0.6	90.7 ± 13.3	16.3 ± 3.4	9.4 ± 0.7
Adult male (*n=10*)	18.2 ± 1.9[a]	21.5 ± 5.0[a]	13.0 ± 1.3[a]	140.3 ± 8.2[a]	9.7 ± 1.7[a]	10.0 ± 1.6
Aged male (*n=11*)	20.9 ± 1.9[a,b]	26.1 ± 3.9[a]	13.9 ± 1.8[a]	157.1 ± 11.2[a,b]	12.1 ± 2.4[a,b]	12.3 ± 1.3[a,b]
MEK	Blood	Liver	Kidney	Fat	Muscle	Brain
PND10 male	207.8 ± 3.0	200.1 ± 8.3	191.0 ± 9.0	214.7 ± 24.5	181.0 ± 6.1	186.2 ± 3.8
PND10 female	205.5 ± 2.6	199.6 ± 13.4	193.1 ± 5.6	215.0 ± 15.3	183.9 ± 11.8	187.9 ± 4.7
Adult male	195.9 ± 7.2[a]	211.1 ± 38.4	242.2 ± 44.1[a]	230.1 ± 12.3	156.4 ± 6.9[a]	157.8 ± 7.2[a]
Aged male	197.3 ± 3.5[a]	245.4 ± 45.9[a]	221.9 ± 31.2[a]	213.1 ± 18.1[b]	158.3 ± 7.1[a]	161.3 ± 9.6[a]
CFM	Blood	Liver	Kidney	Fat	Muscle	Brain
PND10 male	12.8 ± 0.7	17.7 ± 1.3	12.7 ± 0.9	239.3 ± 47.9	28.9 ± 5.8	9.7 ± 0.5
PND10 female	13.0 ± 1.0	17.1 ± 1.0	12.6 ± 0.8	248.1 ± 38.5	32.2 ± 9.9	10.2 ± 0.9
Adult male	19.3 ± 1.9[a]	19.3 ± 3.4	16.4 ± 1.9[a]	384.9 ± 18.2[a]	13.1 ± 2.9[a]	13.7 ± 1.5[a]
Aged male	22.6 ± 1.7[a,b]	27.8 ± 6.1[a,b]	17.1 ± 2.8[a]	436.8 ± 26.4[a,b]	20.0 ± 5.9[a,b]	17.3 ± 1.6[a,b]

[a]Statistically significant difference between adult male or aged male rat and PND 10 male pup ($p \leq 0.05$).
[b]Statistically significant difference between aged male rat and adult male rat ($p \leq 0.05$).

Table 3. Continued

BEN	Blood	Liver	Kidney	Fat	Muscle	Brain
PND10 male	9.7 ± 0.5	16.8 ± 1.5	11.9 ± 0.9	267.9 ± 54.1	31.0 ± 7.9	9.7 ± 0.5
PND10 female	10.0 ± 1.0	16.1 ± 1.2	11.8 ± 0.8	278.2 ± 44.6	33.9 ± 11.3	9.9 ± 1.0
Adult male	13.4 ± 2.1[a]	17.9 ± 3.6	15.7 ± 2.4[a]	426.2 ± 22.2[a]	10.9 ± 3.2[a]	14.5 ± 1.7[a]
Aged male	16.8 ± 1.7[a,b]	27.6 ± 6.7[a,b]	16.5 ± 3.0[a]	494.6 ± 30.6[a,b]	19.2 ± 6.8[a,b]	19.7 ± 1.8[a,b]
TCE	Blood	Liver	Kidney	Fat	Muscle	Brain
PND10 male	13.1 ± 0.8	22.1 ± 2.3	15.2 ± 1.3	398.7 ± 89.2	43.9 ± 11.0	11.0 ± 0.6
PND10 female	13.4 ± 1.8	21.2 ± 1.7	15.0 ± 1.1	424.5 ± 67.5	48.6 ± 17.3	11.6 ± 1.2
Adult male	17.5 ± 3.6[a]	20.5 ± 4.0	17.6 ± 3.9[a]	631.4 ± 43.1[a]	12.6 ± 4.3[a]	17.4 ± 2.6[a]
Aged male	21.8 ± 1.9[a,b]	34.8 ± 8.7[a,b]	19.9 ± 3.4[a]	757.5 ± 48.3[a,b]	26.4 ± 10.3[a,b]	25.0 ± 2.0[a,b]
PCE	Blood	Liver	Kidney	Fat	Muscle	Brain
PND10 male	15.0 ± 1.1	42.2 ± 5.2	32.3 ± 3.6	946.2 ± 164.9	92.9 ± 19.4	25.4 ± 1.0
PND10 female	15.2 ± 1.7	39.8 ± 4.3	30.6 ± 2.3	990.4 ± 150.5	106.3 ± 34.7	26.2 ± 1.6
Adult male	13.6 ± 6.4	35.0 ± 9.5	32.7 ± 10.3	1529.5 ± 192.5[a]	25.0 ± 8.0[a]	40.4 ± 8.0[a]
Aged male	20.9 ± 2.8[a,b]	65.9 ± 17.2[a,b]	37.7 ± 6.0[a]	2002.4 ± 161.1[a,b]	60.4 ± 24.5[a,b]	58.3 ± 3.7[a,b]

[a]Statistically significant difference between adult male or aged male rat and PND 10 male pup ($p \leq 0.05$).
[b]Statistically significant difference between aged male rat and adult male rat ($p \leq 0.05$).

Table 4. Levels of protein, cholesterol and triglycerides in rat serum (mean ± s.d.)

	Total Protein (g/dL)	Cholesterol (mg/dL)	Triglycerides (mg/dL)
PND 10 male	3.7 ± 1.0	124.2 ± 17.7	141.3 ± 35.4
PND 10 female	3.7 ± 0.6	138.6 ± 11.0	129.1 ± 29.9
Adult male	6.3 ± 0.4	83.7 ± 9.9	133.5 ± 83.5
Aged male	6.9 ± 0.5	99.8 ± 13.6	207.9 ± 103.2

Human Partition Coefficients

Table 5 lists the human blood:air PC values for methylene chloride, methyl ethyl ketone, chloroform, benzene, trichloroethylene and perchloroethylene. Compiled data are listed in Appendix A. Both male and female pediatric ages range from 3 to 7 years, while male and female adult ages range from 21 to 87 years. There were few differences in measured PC values between pediatric and adult blood samples. When blood:air PCs were compared individually with age of the donor, chloroform ($r^2 = 0.19$) and trichloroethylene ($r^2 = 0.16$) PCs were significantly, yet minimally correlated with age in males (Figure 1). No other chemical tested displayed a correlation between measured PC and age.

Table 5. Human blood:air partition coefficients (mean ± s.d.)

	DCM	MEK	CFM	BEN	TCE	PCE
Adult						
Male	13.0 ± 1.4	184.6 ± 7.9[a]	13.8 ± 2.0[a]	9.0 ± 1.2	11.7 ± 1.9[a,b]	15.8 ± 3.3
Female	12.5 ± 1.9	186.9 ± 6.6[a]	12.5 ± 2.1	8.3 ± 1.7	10.6 ± 2.3	15.3 ± 3.7
Pediatric						
Male	12.6 ± 1.2	191.3 ± 5.7	12.6 ± 1.4	8.9 ± 1.3	11.2 ± 1.8	15.7 ± 3.3
Female	12.2 ± 1.3	190.2 ± 7.2	12.8 ± 1.2	8.7 ± 1.2	11.0 ± 1.6	15.7 ± 2.9

[a]Statistically significant difference between adult and pediatric blood:air partition coefficient ($p \leq 0.05$).
[b]Statistically significant difference between male and female blood:air partition coefficient ($p \leq 0.05$).

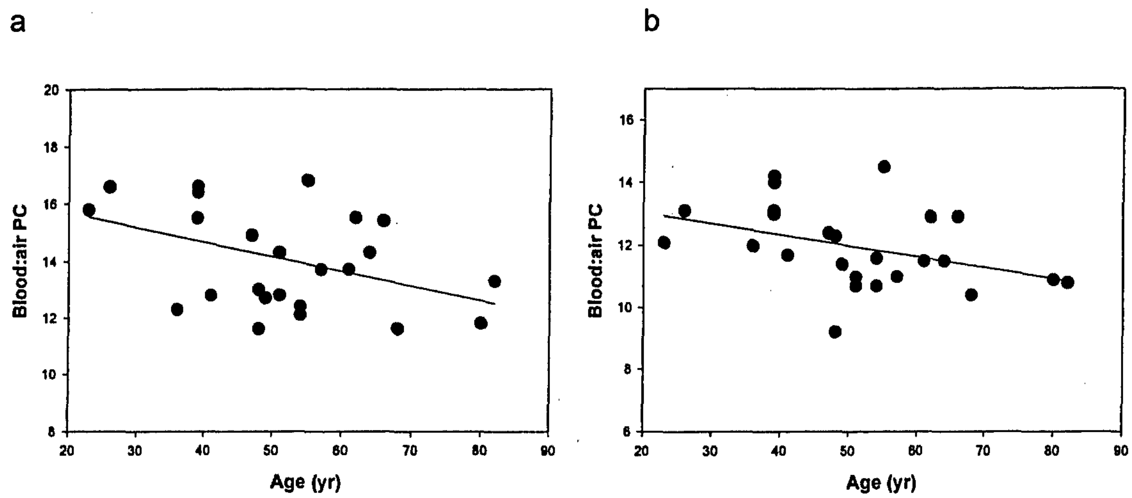

Figure 1. Correlation between measured blood:air partition coefficient and age. a) Adult human male blood:air partition coefficients for chloroform are significantly, yet minimally correlated ($r^2 = 0.19$) with age ($p \leq 0.05$). b) Adult human male blood:air partition coefficients for trichloroethylene are significantly, yet minimally correlated ($r^2 = 0.16$) with age ($p \leq 0.05$).

Total plasma protein levels were essentially the same between pediatric and adult patients (Table 6). However, adult cholesterol and triglyceride levels (male and female combined) were 72% and 90% higher than pediatric levels (male and female combined), respectively. In spite of this significant difference in plasma cholesterol and triglyceride, there was not a corresponding increase in measured PC between pediatric and adult patients for any chemical tested.

Table 6. Levels of protein, cholesterol and triglyceride in human plasma (mean ± s.d.).

	Total Protein g/dL	Cholesterol mg/dL	Triglycerides mg/dL
Adult			
Male	7.3 ± 0.6	209.5 ± 43.8	185.5 ± 84.2
Female	6.7 ± 0.7	209.8 ± 55.6	194.1 ± 96.0
Pediatric			
Male	6.9 ± 0.4	98.5 ± 76.1	91.9 ± 42.6
Female	7.4 ± 0.5	145.1 ± 29.2	107.9 ± 57.8

DISCUSSION

It is widely accepted that chemical-specific PCs should be determined experimentally when possible. Currently, the focus has shifted to reducing errors that result from applying adult PCs to the assessment of risk to elderly and pediatric populations. The purpose of this study was to determine PCs experimentally for selected volatile organic compounds in pediatric, adult and elderly rats and humans. By comparing the measured PCs for the compounds of interest, it can be determined if there are age-dependent differences. The compounds selected, DCM, MEK, CFM, BEN, TCE and PCE, have a range of log K_{ow} values from 0.29 for MEK to 3.4 for PCE, representing fairly water soluble to virtually insoluble compounds.

Overall, S-D rats showed an increase in PC with advancing age for every tissue except muscle. Muscle PCs were consistently lower in adult and aged rats when compared to male PND 10 pups. While it is unclear what is driving the lower PCs in muscle, this difference may suggest a more significant role for protein binding and its effect on measured PCs. Human blood:air PCs exhibited little to no age related differences between the pediatric and adult groups. Within the adult group, only CFM and TCE displayed any correlation between blood:air PC and age; however, the significance of this was slight.

With the exception of MEK, these compounds are highly lipophilic. Because of this property, it is assumed that the amount of fat incorporated into tissue will increase with age and cause a corresponding increase in PC. When compared on a mean basis, higher serum triglyceride levels in rats were correlated with increasing PC values. However, when individual blood:air PC values were compared to the corresponding serum lipid levels, there was no correlation. As suggested by the rat muscle data, it appears that protein binding may play a role in chemical partitioning. However, only rat and human blood:air PCs for TCE showed a slight positive correlation with serum and plasma protein levels, indicating that another possible mechanism may be involved for muscle PCs.

One of the objectives of this research was to measure PCs for these compounds in fresh pediatric and human tissue. Unfortunately, obtaining tissues, especially pediatric, is difficult, and we were unable to complete these measurements. Even though frozen human tissue is somewhat easier to obtain, all PC determinations were done in fresh tissue. To validate the use of frozen tissue for future work, PCs were measured for all compounds in fresh and frozen

tissues harvested from adult male rats. The data were highly variable between fresh and frozen tissues with no observable trend for higher or lower measurements. This suggests that the use of frozen tissues is not recommended for PC determination. The data set for frozen versus fresh tissue is attached as Appendix B.

For measurement of blood:air PCs in rats, blood was collected in a heparinized syringe to prevent coagulation. However, human blood (pediatric and adult) received from the Wright-Patterson Air Force Base Medical Center was collected in EDTA tubes. To eliminate potential differences in measurement of PCs caused by the different anticoagulants, blood:air PCs were determined in rat blood treated with either heparin or EDTA. There were no detectable differences in PCs for the two anticoagulants. The data set for the different anticoagulants is attached as Appendix C.

The PCs reported here represent an initial attempt to uncover age dependent differences in pharmacokinetic parameters. While it is unclear what factors directly affect or alter the PC between groups, there are significant differences in the measured rat PCs for the compounds and tissues tested. Human blood:air PCs, on the other hand, show little to no age dependence.

Concern for representing sensitive subpopulations accurately is justified. It is unknown if human tissue partition coefficients alter with age, as rat tissues have been shown to change during this project. However, this project has shown that human blood:air PCs do not vary significantly with age, at least with this class of chemicals. Therefore, PBPK models using experimentally derived human blood:air PCs can provide reliable predictions of tissue dose across age groups.

REFERENCES

Avery, G. B. 1981. Neonatology: Pathophysiology and Management of the Newborn. Second edition. Philadelphia, JB Lippincott, 1175-1177.

Fisher, J. W., D. A. Mahle, L. Bankston, R. J. Greene, and J. M. Gearhart. 1997. Lactational transfer of volatile chemicals in breast milk. *Am. Ind. Hyg. Assoc. J.* 58(6): 425-31.

Gargas, M. L., R. J. Burgess, D. E. Voisard, G. H. Cason and M. E. Andersen. 1989. Partition coefficients of low-molecular-weight volatile chemicals in various liquids and tissues. *Toxicol. Appl. Pharmacol.* 98(1): 87-99.

Lerman, J., G. A. Gregory, M. M. Willis and E. I. Eger. 1984. Age and solubility of volatile anesthetics in blood. *Anesthesiology.* 61: 139-143.

Ramsey, J. C. and M. E. Andersen. 1984. A physiologically based description of the inhalation pharmacokinetics of styrene in rats and humans. *Toxicol. Appl. Pharmacol.* 73(1): 159-175.

APPENDIX A: COMPILED RAT AND HUMAN PARTITION COEFFICIENT DATA

Table A-1. PND 10 Male Rat Pup Summary

Methylene chloride

	Blood		Liver		Kidney		Fat		Muscle		Brain	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
1	14.9	0.6	13.8	0.1	10.1	0.1	63.9	26.3	23.3	6.4	8.8	0.6
2	14	0.4	12.3	0.1	10.5	0.2	98.8	22.9	14.3	2.7	8.6	0.8
3	14.4	0.1	14	0	9.5		103.5	7	13.5		9.1	0.5
4	16.2	0.1	11.9	0.2	9.8	0	100.4	7.1	16.2	10.2	10.3	5.1
5	14.8	0.1	13.9	0.7	10.5	0.2	78.4	5.8	13.2	2.8	9.2	0.9
6	15.3	0.3	11.8	0.5	9.6	0.7	103.6	3.4	20.8	3.7	8.5	0.2
7	14.2	0.1	11.8	0.3	9.4	0.9	79.1	0.3	15.3	3.1	8.6	0.5
8	14.2	0.2	13.1	0.6	9.6	0.3	52.5	14.6	14.8	3.3	9.1	0.1
9	12.9	0.7	12.6	0.6	9.8	0.4	85.6	4.7	13.7	0.7	8.8	0.3
10	14.3	0.3	13.3	0	10.2	0.4	110.9	5.5	16.9	0.7	10.1	0.1
average	14.5		12.7		9.9		90.3		15.4		9.1	
std dev	0.9		0.9		0.4		18.3		2.4		0.7	

Methyl ethyl ketone

	Blood		Liver		Kidney		Fat		Muscle		Brain	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
1	205	1.1	208.4	44.4	176.8	1.5	173.3	65	190.4	4.5	182.4	3.2
2	205.6	0.1	200.8	9.9	208.3	8.8	217.3	24.4	186	42.8	183.7	4.1
3	214	0.9	202.7	13.2	196.8		258.4	6.5	182.3		193.1	4
4	210.8	0.5	206.5	8.5	190.6	3.9	234.5	17.4	357	233.7	118.5	75
5	208.3	2.4	211.2	20.9	196.1	0.6	213.3	14.7	180.5	1.9	190.1	5.1
6	207.6	1.8	201.1	22.9	187.3	8.4	236.1	32.4	180.7	2.9	181.8	4.3
7	204	0.3	199.3	22.6	189.8	10.6	199.1	4.3	176.8	1.6	188.5	4.2
8	207	4.7	202.5	6	187.5	8.1	183	9.7	186.6	17.6	187.5	0.5
9	208.3	1	183.9	12.1	192.6	7.7	181.9	6.1	172.4	6.8	184.2	0.2
10	210.3	0.6	190.7	2	183.4	5.3	224.2	8.9	173	0.9	184.6	0.7
average	208.1		200.7		190.9		212.1		198.6		179.4	
std dev	3.0		8.1		8.5		27.5		56.0		21.7	

Chloroform

	Blood		Liver		Kidney		Fat		Muscle		Brain	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
1	13.5	0.5	18.2	1.1	13.8	0.1	174.6	68.4	53.7	19.2	9.5	0.6
2	12.5	0.3	16.2	0.4	13.8	0.7	274	62.8	26.6	8	9.1	0.7
3	13.1	0	18.7	0.1	11.3		285	17.5	23.9		9.8	0.5
4	14.4	0.1	17.5	0.6	12	0.2	273.3	15.2	17.8	11.2	18.7	8.7
5	13.3	0.2	20.4	0.6	13.5	0.2	215	20.5	22.5	9.5	10	0.9
6	13.6	0.3	16.7	0.8	12.2	0.7	283.7	6.5	42.5	11.2	9.4	0.3
7	12.5	0	16.4	1	12	1	218.2	6.3	27.5	6.2	9.3	0.5
8	12.6	0.3	16.7	0.5	12.4	0.2	138.2	38.1	26.4	7.7	9.8	0.1
9	11.6	0.7	17.6	0.8	12.3	0.4	237.8	11.4	25.1	1.8	9.7	0.2
10	11.9	0.1	17.8	0	12.3	0.8	278.7	14.6	33.3	2.7	11	0
average	12.9		17.6		12.6		237.9		29.9		10.6	
std dev	0.8		1.3		0.8		51.0		10.7		2.9	

Table A-1. PND 10 Male Rat Pup Summary (continued)

Benzene

	Blood		Liver		Kidney		Fat		Muscle		Brain	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
1	10.2	0.6	16.7	0.2	12.9	0.1	191.9	77.4	57.6	21.9	8.9	0.8
2	9.5	0.4	15.5	0.1	13.3	0.8	309	72.8	28	8.7	8.9	0.7
3	10	0.1	18.1	0.4	10.5		321.4	19.3	24.6		9.3	0.4
4	11.2	0.1	16.7	0.1	11.2	0.1	305.5	16.4	16.8	10.6	19.1	8.7
5	10	0.2	20.2	1.6	12.6	0.3	239.9	22.9	22.9	10.7	9.6	0.9
6	10.2	0.1	16	0.8	11.3	0.7	317.8	6.7	45.1	12.4	8.9	0.3
7	9.5	0	15.2	0.4	11.3	1	243.6	7.6	28.5	7	8.8	0.6
8	9.5	0.2	15.8	0.5	11.6	0.1	154.7	42.8	26.9	7.8	9.4	0.1
9	8.7	0.6	16.9	0.9	11.6	0.4	267.6	13.8	25.6	1.6	9.4	0.1
10	9.3	0.1	16.8	0.1	11.5	0.8	310	16.4	34.9	2.7	10.7	0
average	9.8		16.8		11.8		266.1		31.1		10.3	
std dev	0.7		1.5		0.9		57.9		12.0		3.1	

Trichloroethylene

	Blood		Liver		Kidney		Fat		Muscle		Brain	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
1	13.7	0.8	21.2	0.5	16.7	0.6	292.6	11.8	83.8	35.9	10.3	0.9
2	13.1	0.4	20.2	0.8	17.2	1.3	462.5	127.9	41.4	13.9	10.7	0.8
3	13.4	0.1	24.7	1	13.3		492.1	28.9	34.5		10.7	0.2
4	15	0.1	21.9	0.3	14	0.2	465.8	23.2	19.6	12.4	27.1	11.9
5	13.9	0	26.7	2	16.3	0.3	368.5	32.5	31.8	15.8	11.2	0.9
6	14	0.4	20.4	0.6	14.5	0.8	483.3	4.1	64.2	19.8	10.6	0.5
7	13.4	0	20	0.1	14.3	1.1	374.6	13.8	39.8	8.7	10.5	0.5
8	12.8	0.1	20.2	0.8	14.9	0	236.5	64.4	37.8	12.2	11	0.1
9	11.6	1	23	1.3	15	0.4	412.2	20	38.5	2.7	11.1	0.3
10	11.9	0.2	22.3	0.1	14.2	0.9	465.5	34	48.6	1.8	12.5	0.3
average	13.3		22.1		15.0		405.4		44.0		12.6	
std dev	1.0		2.2		1.3		86.7		18.1		5.1	

Perchloroethylene

	Blood		Liver		Kidney		Fat		Muscle		Brain	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
1	15.5	1	39.5	2.8	39.1	2.9	733	277.7	178.2	97.3	23.8	0
2	15.6	0.5	36.7	1.9	34.7	2.2	1033.1	389.7	98.6	38.3	25.2	0.6
3	15.2	0	49.3	4.1	28.3		1070.3	26.9	74.2		24.7	0
4	16.3	0	42.1	0.7	28.2	0.4	1130.6	63.7	46.3	30.3	60.4	28.3
5	15.3	0.1	49.2	4.1	34.8	1.2	905	62.8	64.9	32.2	25.7	1.3
6	15.4	0.5	39	0.1	30.9	0.9	1190.6	50.2	128	35.8	25.7	1.1
7	16.8	0.2	38.1	3.6	29.2	0.9	880.4	28.5	82.9	13.2	24.8	1
8	13.9	0.1	37.7	1.6	33.3	1.5	592.8	144.7	84.9	34.5	25.2	0.3
9	13.7	0.7	47.9	3.4	31.2	1	998.7	59.7	88.2	7.9	25.9	0.9
10	13.4	0.1	42.3	0.1	28.6	1.9	915.2	48.7	105.2	2.3	27.3	1
average	15.1		42.2		31.8		945.0		95.1		28.9	
std dev	1.1		4.9		3.6		181.6		36.7		11.1	

Table A-2. PND 10 Female Rat Pup Summary

Methylene chloride

	Blood		Liver		Kidney		Fat		Muscle		Brain	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
1	14.5	0.6	12.6	1.5	10.1	0.3	83.3	8.4	20.4	0.6	9.2	0.7
2	15.7	1	11.2	0.3	9.3	0.4	104.8	7.3	45.1	32	8.4	0.6
3	14	0.5	13.9	0.2	10.8	0.4	10.6	5	15.9	2.3	9.9	1.2
4	16.1	0.9	13.2	0.4	11.3	1.7	105.3	15.9	16.8	10.7	10.5	6.8
5	13.9	1.2	12.2	0.2	10.4	0.1	91.5	1.4	12.7	0.8	9.1	0.1
6	15.5	0.3	11.4	0.5	9.3	0.2	93.8	5.9	13.9	1.7	9.3	0.4
7	14.5	0.4	11.9	0.3	9.3	0.4	83.7	5.2	13.3	1.9	9.1	0.2
8	14.1	0.2	12.1	0.1	10	0.3	66.8	13.2	14.3	2	9.5	0
9	13.6	0.5	12.2	0.5	9.9	0.1	99.1	1.5	16	0.9	8.8	0.6
10	14	0.3	13	0.3	10.6	0.5	82.7	1.5	18	2.9	10.8	1.2
average	14.6		12.3		10.1		82.0		18.4		9.5	
std dev	0.9		0.9		0.7		29.4		10.1		0.8	

Methyl ethyl ketone

	Blood		Liver		Kidney		Fat		Muscle		Brain	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
1	203.9	4.3	224.4	68.5	193.4	7	203.9	31.4	197.3	13.2	180.4	3
2	202.4	6.5	205.6	25.3	198.5	1.1	215.6	8.4	206.6	22.3	183.9	0.5
3	210.8	3.1	201.4	15.2	195	2	246.7	4.5	188.5	8.5	189.4	0.5
4	208	4.2	218.2	31.5	185	0.6	230.7	29.1	384	269.9	116.1	78.7
5	206.9	1.7	192.2	1.1	203.1	4	222.4	4.8	178.3	3.3	187.6	3.1
6	206.2	0.3	190.2	11	187	1.4	217.2	13.1	169.1	1.4	188	1.8
7	204.5	7	184.1	0.3	185.8	1.5	214.7	10.4	175.4	4.5	195.6	11.9
8	206.3	4.6	204.3	19.3	189.4	5.4	199.7	4	181.2	2.3	192	1.9
9	202.5	2.9	183.4	7.9	189.7	11.4	220.2	6.5	175.4	7.9	183.5	3.7
10	205.6	2.2	210.6	6	195.3	11.9	194.6	2.3	182.9	6.7	190.7	0.4
average	205.7		201.4		192.2		216.6		203.9		180.7	
std dev	2.6		14.0		5.9		15.2		64.3		23.1	

Chloroform

	Blood		Liver		Kidney		Fat		Muscle		Brain	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
1	13.2	0.6	17.7	3.4	13.9	0.4	232.2	8.9	43.5	2.2	9.9	0.7
2	15	1.5	15.5	0.4	12.2	0.4	292.5	25.1	119.3	96	9	0.6
3	12.6	0.5	18.5	0	12.7	0.4	299.7	10.3	31	6.3	10.9	1.5
4	14.3	0.9	19.1	0.5	13.8	1.5	287.7	42.4	18.7	12	19.2	14.8
5	12.2	1.5	17.7	0.4	12.9	0.1	249.2	5.1	20.5	2.7	10	0.2
6	14.4	0.2	15.9	0.7	11.7	0.1	256.9	11.4	25.5	4	10.1	0.4
7	12.9	0.7	16.5	0.3	11.5	0.6	232.2	11.9	24.1	4.4	9.9	0.3
8	12.7	0.3	16.5	0.1	12.3	0.2	177.8	36.9	25.9	6.1	10.2	0.1
9	11.9	0.6	17.3	0.8	13.1	0.1	275.2	4.2	32.9	1.2	9.8	0.7
10	12.2	0.3	18.1	0	13	0.4	217	3.7	34.7	8.9	12.1	1.5
average	13.1		17.3		12.7		252.0		37.6		11.1	
std dev	1.1		1.2		0.8		38.4		29.6		3.0	

Table A-2. PND 10 Female Rat Pup Summary (continued)

Benzene

	Blood		Liver		Kidney		Fat		Muscle		Brain	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
1	9.9	0.7	15.6	1	13	0.4	256.9	10.9	45.6	2.6	9.3	0.8
2	11.9	2.2	14	0	11.6	0.4	331.1	28.8	133.7	109.6	8.7	0.6
3	9.6	0.5	17.4	0.1	11.9	0.4	337.9	12.4	32.8	7.1	10.8	2.1
4	11.1	0.9	17.9	0.1	13.1	1.5	322.7	47.5	17.8	11.4	19.8	15.8
5	9.3	1.2	17.4	0.4	12.2	0.1	279.3	5.2	20.6	3.1	9.6	0.2
6	11.4	0.8	15.2	0.7	10.8	0.1	286.8	12.6	26.3	4.3	9.6	0.4
7	9.7	0.5	15.8	0.4	10.6	0.7	259.6	12.7	24.8	4.9	9.4	0.2
8	9.6	0.3	15.2	0.1	11.5	0.2	198.6	41.5	26.9	7	9.8	0.1
9	9	0.4	16.9	0.9	12.5	0.2	310.6	4.9	35.1	1.1	9.6	0.6
10	9.3	0.3	17	0.1	12.4	0.4	242.7	3.9	36.6	10.1	12.1	2
average	10.1		16.2		12.0		282.6		40.0		10.9	
std dev	1.0		1.3		0.8		44.3		33.9		3.3	

Trichloroethylene

	Blood		Liver		Kidney		Fat		Muscle		Brain	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
1	13	0.9	21	2.4	16.6	0.6	392	11.8	64.2	0.8	10.6	0.9
2	17.2	4.4	18.6	0.2	14.8	0.5	503.2	50.5	201.8	166.4	10.2	0.4
3	12.8	0.3	23.6	0.6	14.6	0.4	511.9	11.1	47.5	10.7	12.7	2.7
4	14.4	0.8	23.1	0.5	16.1	1.4	493.3	71.7	20.9	13.6	28.1	23.2
5	12.3	1.8	22.8	0.8	15.3	0.1	427.3	6.8	28.1	4.4	11.1	0.3
6	15.5	1.6	19.1	1.1	13.7	0.1	437.7	17.2	36.8	5	11.2	0.4
7	13.6	0.8	20.7	0.3	13.2	0.7	399.3	18.2	35.2	8.2	11.2	0.1
8	12.6	0.7	20	0.4	14.4	0.2	303	64.5	37.9	11	11.5	0.2
9	11.8	0.7	22.3	1.5	16.3	0.2	477.5	7.6	51	1.2	11.5	0.7
10	11.8	0.6	22	0	15.4	0.3	368.7	5	52.9	15.5	14.1	2.2
average	13.5		21.3		15.0		431.4		57.6		13.2	
std dev	1.7		1.7		1.1		67.2		52.2		5.3	

Perchloroethylene

	Blood		Liver		Kidney		Fat		Muscle		Brain	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
1	14.8	1.1	36.2	4.4	34.5	2.9	976	38.7	132	3.2	24.4	0.9
2	22.3	9.4	33.4	0.5	29.9	0.5	1093.8	161.7	436.7	368.3	24.1	0.7
3	15.2	0.1	46.3	2.2	30.6	0.8	1155	134.6	110.6	23.6	27.5	3.7
4	15.2	0.5	41.8	3.2	31.4	0.5	1229.5	184	49.8	34.1	61.5	54
5	14.2	1.7	44	1.4	29.3	0.8	995.1	27.9	62.4	5.8	25.6	0.4
6	18.6	5.3	37.5	1.8	28	0.3	1064.2	31.3	78.6	5.3	26.2	0.5
7	16.7	0.3	39.6	0.7	28	0.5	966.3	31.3	82.2	25.6	26.5	0.8
8	14.5	1.1	37	2.4	29.5	0.1	697.3	178.8	81.7	23.1	26.1	0.3
9	13.6	0.5	44.3	3.4	34	0.5	1142.5	41.2	115	4.5	26.1	1.6
10	13.1	0.8	39.7	0.1	31	0.1	822.7	17.1	117.6	35.5	29.4	2.2
average	15.8		40.0		30.6		1014.2		126.7		29.7	
std dev	2.8		4.1		2.2		160.8		112.0		11.3	

Table A-3. Adult Male Rat Summary

Methylene chloride

	Blood		Liver		Kidney		Fat		Muscle		Brain	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
1	17.9	0.3	20.3	2.1	14.5	2.7	143.2	2.2	9.5	2.3	10.7	0.6
2	17.1	1	23.7	0.8	18.9	7.9	130.3	3.5	8.6	1.8	7.7	2.2
3	15.7	0.9	22.3	0.5	12.2	2.3	150.4	22.3	8.9	0.3	12.1	3.1
4												
5												
6	10.4	6.3	17.6	12.6	15.6	3.4	58.3	34.7	48.3	37.4	10.6	2.8
7	18.1	2.4	30.5	10.5	12.8	1.1	130	2	7.3	1	8	2.2
8	17.2	0.7	17.7	2.2	10.9	1.7	138.7	6.1	10.4	2.9	8.8	0.6
9	18.1	0.5	13.4	0.4	13.8	2.1	148.9	5	13.1	0.5	10.3	1.7
10	21.9	0.3	20.1	0.5	11.9	0.6	134	11	9.3	0.8	11.2	0.6
11	19.6	0.8	23.7	0.3	12.8	0.3	146.8	4.7	10.3	0.7	10.8	1
average	17.3		21.0		13.7		131.2		14.0		10.0	
std dev	3.1		4.8		2.4		28.4		13.0		1.5	

Methyl ethyl ketone

	Blood		Liver		Kidney		Fat		Muscle		Brain	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
1	194.9	1.6	260.7	34.2	210.7	8.8	232.9	5	166.4	4.7	159.3	0.3
2	189.3	1.9	197.5	2.6	330.3	36.9	217.1	3.8	155.9	1	151.3	6.5
3	192.5	0.6	277.6	45.6	286.9	20.6	239.1	24.2	156.9	7.5	155.8	2.3
4												
5												
6	151.3	6.6	167.8	23.1	187.4	21.8	127.1	32.4	129.1	17.4	121.4	1.5
7	189.4	3.3	184.9	14.1	213.9	10.8	206.6	8.7	143.8	2.3	149.5	2.4
8	191.3	5.2	187.9	25	200.9	8.4	233.1	9.7	149.8	6.3	151.2	1.6
9	196.4	3.3	167.2	15.6	231.1	22.9	238.5	7.3	159	3.7	161.1	7
10	206.6	3.6	200	8.6	233.7	41	230	18	158.6	4.8	170.5	2.3
11	207.1	6	212.7	24.6	230.3	9.8	243.3	8.2	160.8	3.1	163.6	2.6
average	191.0		206.3		236.1		218.6		153.4		153.7	
std dev	16.3		38.7		45.1		36.2		11.2		13.9	

Chloroform

	Blood		Liver		Kidney		Fat		Muscle		Brain	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
1	18.1	0.3	18.4	2.3	18	2.1	390.5	10.8	11.6	2.7	14	0.7
2	18.7	0.8	18.5	2	18.8	6.4	376.3	11.7	11.4	1.6	11.4	2.5
3	16.2	1.2	19	0.4	12.9	2.7	389.4	28.6	10.8	0.5	15.2	2.5
4												
5												
6	13.1	5.6	14.5	9.2	15.1	2.7	244.6	39.9	40.7	28.9	12.8	1.5
7	19.7	2.2	27.4	7.7	15.8	1.2	349.4	16.3	10.1	0.4	11.9	3.1
8	18.6	0.5	17.4	2.5	14.9	2	390	15.3	14.6	3.8	13.2	0.7
9	19.6	0.5	16.6	0.8	17.3	1.3	403.9	10.4	18.9	1.1	13.6	2
10	22.1	0.1	17.3	0.3	16	0.1	374	30.5	12.7	2.1	15.4	0.6
11	21.3	1.1	19.4	0.5	17.1	0.5	405.6	9.3	14.4	0.4	14.8	1.3
average	18.6		18.7		16.2		369.3		16.1		13.6	
std dev	2.7		3.6		1.8		49.8		9.6		1.4	

Table A-3. Adult Male Rat Summary (continued)

Benzene

	Blood		Liver		Kidney		Fat		Muscle		Brain	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
1	12.7	0.3	16.6	3	17.4	2.1	434.3	12.1	9.7	3.8	14.8	1
2	13.2	0.9	18.1	2.8	18.6	6.8	422.2	13.7	9.5	1.5	12.3	3.1
3	10.2	1.5	16.2	0.6	10.7	3.3	420.8	29.9	7.5	0.2	15.2	2.1
4												
5												
6	7.8	5.7	12.4	8.8	13.2	2.6	265.2	39.7	39.9	30.5	12.2	1.3
7	13.5	2.3	26.3	7.9	14.5	2	381.2	18.2	7.4	0.5	12.2	4.3
8	11.9	1	15.9	3.2	14.4	2.2	426	18.3	12	3.8	14.2	1.2
9	12.8	0.4	14.5	1.2	16.4	1.1	441.1	12.5	17	1.9	13.7	2.5
10	16.7	0.1	16.6	0.2	15.9	0.2	424.2	34.3	11.1	2.4	16.9	0.7
11	16.2	1.1	18.8	0.5	17.2	0.5	459.8	11	13.1	0.6	16.4	1.7
average	12.8		17.3		15.4		408.3		14.1		14.2	
std dev	2.7		3.9		2.4		57.6		10.1		1.8	

Trichloroethylene

	Blood		Liver		Kidney		Fat		Muscle		Brain	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
1	16.8	0.2	20.1	3.9	19.5	1.7	652	20.6	11.4	5.5	17.7	1.3
2	19.1	0.9	23.7	4.3	20.7	6.5	643.8	20.7	11.4	1.4	15.8	3.5
3	12	1.9	16.2	1.1	9.4	3.9	593.7	35.2	6.3	0.2	16.2	1.7
4												
5												
6	11.7	5.2	14	6.5	12.2	2.2	419.5	45.1	31.2	23	12.5	0.7
7	17.8	2.3	27.1	6.5	15.8	3.8	559.8	30	8.3	0.8	14.6	5.6
8	14.9	1.1	17.6	4.4	15.8	2.7	616.7	28.4	13.2	4.5	17.3	2.4
9	14.8	0.4	15.5	1.9	17.9	0.6	628.8	18.1	19.5	4.6	14.7	3.1
10	21.3	0.7	20.6	0.5	20.3	1	652.4	52.9	14	3.6	21.5	0.7
11	23.1	1.6	23.3	0.6	21.2	0.6	703.7	16.7	16.9	0.5	21	2.3
average	16.8		19.8		17.0		607.8		14.7		16.8	
std dev	3.9		4.4		4.1		81.3		7.4		3.0	

Perchloroethylene

	Blood		Liver		Kidney		Fat		Muscle		Brain	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
1	14.6	0.3	37.7	6.7	38.5	1.3	1645	65.3	23.2	8.8	43.7	1.9
2	17.8	0.8	47.8	8.1	35.5	9.6	1645.7	49.9	24.9	4.4	42.2	4.1
3	5.2	2.3	23.2	3.1	14.2	4.2	1328.2	69.2	10.4	0.6	31.9	3.4
4												
5												
6	6.6	.4	21.2	4.6	13.9	2.6	1032.7	87	25.6	14.3	23.5	0.6
7	13.1	2.2	37.1	7.4	28.9	8.3	1344.5	73.9	19.1	3.2	36.8	8.1
8	8.3	1.4	27.1	8.1	28.1	4.2	1390.9	74	23.7	6.6	38	5.8
9	6.9	0.3	23.2	3.2	27.3	2.8	1364.8	33.8	33.3	13.6	29.2	3.9
10	19.3	0.9	39.1	1.6	45.2	4.5	1701.5	145.1	29.7	7.7	51.5	1.8
11	23.3	1.8	45.2	1.3	44.2	1.4	1815.7	48.4	35.5	1.8	50.3	3.6
average	12.8		33.5		30.6		1474.3		25.0		38.6	
std dev	6.4		10.0		11.5		244.7		7.5		9.4	

Table A-4. Aged male rat summary

Methylene chloride

	Blood		Liver		Kidney		Fat		Muscle		Brain	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
1	21.2	1.2	23.6	0.8	13.2	0.2	149.7	5.7	11.5	1.2	13.1	1.2
2	19.6	1.8	19.6	0.8	13.4	0.3	140	10.7	12	1.2	10.7	2.5
3	16.7	1.2	29.7	0.7	10.2	0.6	134.9	5.6	7.9	1.6	9.3	1.3
4	20.4	0.5	30.3	2.6	14.4	0.5	158.7	8.8	11.4	0.5	13.1	1.7
5	22.6	0.9	21.7	0.9	12.9	0.5	163.8	7.9	12.1	0.7	14.2	0.7
6	23.7	1.1	29.4	1.5	15.3	0.3	169.1	5.9	15.4	0.5	13	0.9
7	19	1.1	23.8	2	13.5	0.8	154.7	8.7	9.7	0.4	12.2	0.9
8	21.5	0.6	29.8	0.5	14.5	0.4	162	20.7	10.5	0.1	12.5	0.3
9	22	0.2	24.7	1.5	17.4	1.2	163.5	4	13.7	1.9	12.6	0.7
10	21.8	0.8	30.5	1.5	14	0.5	166.9	4.7	13.9	0.5	12.5	0.1
11	21.8	0.7	24.2	0.5	14.3	0.6	164.5	5.9	15.5	1.7	12.3	0.2
average	20.9		26.1		13.9		157.1		12.1		12.3	
std dev	1.9		3.9		1.7		11.2		2.3		1.3	

Methyl ethyl ketone

	Blood		Liver		Kidney		Fat		Muscle		Brain	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
1	201.3	3.8	222.7	16.3	205.4	8.1	201.6	4.4	160.6	3	162.3	5.8
2	193.1	4	212.4	18.9	230.3	9.1	197.1	16.6	160.6	5.1	143.4	29
3	165.5	6.7	177.2	17	179.8	8.4	170.9	8.5	137.5	1.6	141.9	0.9
4	193.7	3.8	270.6	13.1	221.3	10.9	210.2	12.3	160.8	1.3	162.3	4.3
5	203.4	6.5	207.6	16.9	190.1	3.1	229.5	7.7	157.1	2.1	170.7	4.9
6	195.8	5.4	232.2	6.7	212.8	6.3	229.2	6.8	161.6	2.4	165.4	1.7
7	197	1.3	239.1	27.6	206	7.2	208.2	13.3	157.9	2.4	161.8	2.9
8	199.7	4.9	303.6	84.1	250.5	28.2	225.5	31.9	162.5	2.2	168.6	1.8
9	199.5	5.3	299.9	81.3	292.3	30.2	228.5	4.2	162.3	1.4	167.1	5.7
10	194.6	4.8	215.5	64	210.9	12.4	223.7	5.2	159.9	3	165.5	2.6
11	194.9	5.9	379.6	112	241.7	9.5	219.7	9	161	2.5	164.9	1.1
average	194.4		250.9		221.9		213.1		158.3		161.3	
std dev	10.1		57.8		31.2		18.1		7.1		9.6	

Chloroform

	Blood		Liver		Kidney		Fat		Muscle		Brain	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
1	23	0.7	24.4	0.8	15	0	415	18.7	17.1	3.2	18.2	1.8
2	22.4	1.6	20.7	0.2	15.5	0.3	391.4	28.9	19.7	3.3	15.6	3.7
3	19.3	1	20.9	1.3	12.2	0.8	395.6	9.8	13.3	3.7	13.9	1.8
4	22.4	0.4	32.8	3.2	18	0.3	439.2	26.4	16.4	1	18.2	2.9
5	24.3	0.9	23.2	2.5	16.2	0.9	451	19.7	18.4	1.6	20	1.2
6	24	1.5	30.8	1.2	19.6	0.4	465.3	20.7	28.5	1.6	18.4	0.9
7	19.4	0.5	23.5	2	16.2	1	423.6	27	12.7	0.6	16.7	1.4
8	23.3	0.6	36.2	2.7	18.4	1.2	450.8	59.9	15.3	0.2	17.4	0.4
9	23	0.7	24.2	2.5	23	3	453.7	9.6	24.4	6	17.6	1
10	23.7	1.1	37.5	4.7	16.4	0.6	464.5	11.8	23.7	1.5	17.3	0.1
11	23.3	0.8	31.1	1.6	18	0.7	454	16.2	30.2	4.5	17.5	0.3
average	22.6		27.8		17.1		436.7		20.0		17.3	
std dev	1.7		6.1		2.8		26.4		5.9		1.6	

Table A-4. Aged male rat summary (continued)

Benzene

	Blood		Liver		Kidney		Fat		Muscle		Brain	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
1	17.5	0.9	24.1	0.8	14.4	0.2	467.2	20.2	15.8	3.2	18.2	1.8
2	17.1	1.5	19.9	0.4	14.5	0.1	443.1	36.7	19	3.5	17.7	4.1
3	13.1	1	20.7	1.3	11.5	0.6	451.1	14.8	12.1	4.2	16.1	2.2
4	16.8	0.5	33.2	3.5	17.7	0.2	499.3	28.3	15.1	1.2	20.8	3.9
5	18.2	0.9	22.5	2.9	15.3	0.9	513.6	25.2	17.4	2	22.6	2.1
6	18.3	1.3	31.1	1.4	19.1	0.5	530.3	23.8	29.1	1.8	21.1	1
7	13.7	0.6	22.6	2.1	14.8	0.9	472.3	28.9	10.6	0.9	18.3	1.7
8	17.6	0.6	37.2	3.4	18.1	1.4	514.3	68	14.1	0.2	19.4	0.5
9	17.1	0.5	23.5	3.4	22.6	3.3	509.9	12.1	24.2	6.9	20	1.1
10	17.6	1.3	38.1	5.9	15.5	0.7	524.3	14.8	23.1	1.8	19.3	0.2
11	17.4	0.7	30.8	2	17.6	0.8	515.1	18.9	31	5	19.8	0.5
average	16.8		27.6		16.5		494.6		19.2		19.4	
std dev	1.7		6.7		3.0		30.6		6.8		1.8	

Trichloroethylene

	Blood		Liver		Kidney		Fat		Muscle		Brain	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
1	22.2	0.8	30.5	1.4	17.5	0.5	712.7	31.1	20.9	5.3	26.7	3.3
2	22.2	1.7	24.4	0.6	17.7	0.4	682.5	52.9	26.3	5.3	22.6	4.8
3	19.9	1.1	28.5	2.7	15.1	0.5	691.7	19.2	17	6	21.7	3
4	21.9	0.8	42.2	4.8	20.9	0.9	767.3	45.6	19.6	1.3	26.7	4.7
5	22.5	1.5	26.5	4.5	19.1	1	788.8	39.5	23.4	3.2	27.8	3.9
6	23.5	2.4	40.7	2.7	23.1	2.1	812.7	38.4	40.9	2.5	26.8	0.6
7	16.9	0.1	27.6	2.1	16.5	0.8	710.1	43.4	12.6	1.3	22.6	2
8	23.4	0.7	47.8	6.4	21.1	2	789.6	105.1	18.9	0.9	24.7	0.5
9	21.6	1.3	28.9	4.5	27.1	4.7	778.7	20.2	34.1	10.7	25.3	1.1
10	22.8	2.2	47.9	9.8	18.9	1.6	805.7	23.2	32.2	2.8	24.4	0.3
11	22.3	1.4	38.1	4	21.8	0.9	792.2	29.2	44.8	7.6	25.9	0.9
average	21.7		34.8		19.9		757.5		26.4		25.0	
std dev	1.9		8.7		3.4		48.3		10.3		2.0	

Perchloroethylene

	Blood		Liver		Kidney		Fat		Muscle		Brain	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
1	20.5	0.9	59.6	2.5	34.9	1.3	1865.1	99.1	46.2	12.1	63.3	2.1
2	24	1.6	44.7	3.3	32.2	1.5	1798.6	153.9	57.6	11.7	54	7.7
3	19.9	1.5	56.5	4.8	31.2	1.5	1825.4	61.9	43.4	14.6	55.5	2.9
4	21.1	0.8	81.9	8.1	38.9	3.4	2014.7	100.4	41.5	1.2	60.5	1
5	20.1	1.1	46.6	9.9	36.6	1.4	2064	94.1	52.9	7.7	54.3	9.2
6	23.8	2.1	78.8	5.7	43.5	8.1	2144.1	89.5	89.8	2	61.8	5.2
7	14.1	0.4	51.7	4.6	29.4	1.7	1807.6	118	27.6	2.7	55.9	3.5
8	24.5	0.9	92.5	17.3	38.8	2.8	2299.1	415.5	42.8	2	57.1	3.2
9	19.6	1.7	53.8	6.1	50.2	11.2	2015.9	116.5	79.7	26.3	61.1	4.2
10	21.5	2.8	88.8	20.3	37.1	6.3	2102.2	88.8	74.4	8.6	54.7	2
11	21.3	1.7	69.8	12.1	42.4	1.6	2089.2	80.7	108	18.8	62.9	4.9
average	20.9		65.9		37.7		2002.4		60.4		58.3	
std dev	2.9		17.2		6.0		161.1		24.5		3.7	

Table A-5: Pediatric Human Blood Summary

Male

age (yr)	Methylene chloride mean	s.d.	Methyl ethyl ketone mean	s.d.	Chloroform mean	s.d.	Benzene mean	s.d.	Trichloroethylene mean	s.d.	Perchloroethylene mean	s.d.
3	11.5	0.4	194.3	1.6	12.4	0.4	9	0.3	11.3	0.4	16.5	0.5
4	13.5	0.8	195.2	0.8	13.8	1.4	11.1	2	15	3.4	23.6	7.6
4	11	0.2	193.6	3.9	11.4	0.3	7.8	0.2	9.7	0.3	13.1	0.5
4	14.0	1.9	192.6	1.7	14.4	2.0	10.3	2.0	13.1	2.0	17.3	2.2
4	13.6	1.2	188.3	3.6	12.8	0.9	9.3	0.8	11.6	1.1	16.2	0.9
4	13	0.1	198.8	1.2	13.9	0	10.1	0.1	12.4	0	18.5	0
5	11.1	0.8	184.7	2.1	11	0.5	7.5	0.2	10	0.3	14.7	0.3
5	11.4	0.6	184.4	0.3	10.9	0.2	7.3	0.2	9.9	0.4	14.6	0.6
5	13.1	0.1	197.6	1.4	13	0	9.4	0	11.9	0.1	17	0.6
5	12	0.5	190.9	4.7	12.1	0.5	8.5	0.5	10.6	0.5	14.7	0.6
5	12.4	0.3	182.5	2.8	14.0	0.3	8.4	0.3	10.8	0.4	14.8	0.5
5	13.6	0.14	186.8	1.92	14.2	0.18	9.1	0.28	11.6	0.22	16.4	0.41
5	11.5	0.4	182.5	4.0	11.7	0.1	7.3	0.0	8.3	0.1	11.7	0.1
6	12.5	1.3	192.5	4.1	11.4	0.7	7.9	0.6	10.4	1	15	2.1
6	14	1	194.7	6.4	14.3	0.8	11.7	0.5	14.8	1	22.4	5.4
6	11.6	0.4	196.9	4.2	14	0.4	8.8	0.3	10.8	0.4	14.8	0.5
6	10.3	1.6	184.2	3.5	10.8	2.2	7.5	3.2	9.2	4.6	11	6
7	13.4	0.3	199.8	3.2	13.1	0.3	9.4	0.2	12.1	0.3	17	0.5
7	13.8	4	189	7.1	9.9	2.8	7.2	3.4	7.9	5.5	9.8	6
7	13.8	0	197.1	2.2	13.8	0.1	9.5	0	11.7	0	15.3	0.2
average	12.6		191.3		12.6		8.9		11.2		15.7	
std dev	1.2		5.7		1.4		1.3		1.8		3.3	

Female

age (yr)	Methylene chloride mean	s.d.	Methyl ethyl ketone mean	s.d.	Chloroform mean	s.d.	Benzene mean	s.d.	Trichloroethylene mean	s.d.	Perchloroethylene mean	s.d.
3	12.8	0.4	189.5	2.3	11.9	1.8	8.8	1.9	11.4	2.6	16	3.9
3	13.4	0.5	190.9	0.8	12.2	2.5	8.8	2.4	11.3	2.9	15.9	4
3	11.9	0.1	193.7	2.6	12	0.1	8.8	0.2	10.8	0.3	15.4	0.6
3	10.3	0.2	178.8	5.5	9.1	0.3	5.5	0.3	6.6	0.2	8	0.3
3	11	0.4	170.8	3.2	11.8	1.4	7.5	0.2	9	0.6	12.5	0.7
3	13.5	0.2	201.7	6.1	13.2	0	8.9	0.1	10.7	0	13.9	0.2
4	13.1	1.6	192.9	4.1	13.4	2	10.2	2.7	13.1	4.2	19.3	8.1
4	12.4	0.2	190.4	0.2	12.7	0.2	9.1	0.2	11.6	0.1	16.5	0
4	12.8	0.3	187.8	3.4	15.5	0.3	9.6	0.2	11.7	0.4	16.3	0.4
5	12.4	1.5	189.8	0.1	11.5	0.5	7.9	0.3	10.5	0.4	15.6	0
5	13.3	0.6	188.9	2.5	16.9	0.7	9.9	0.6	13.1	0.7	21	1
5	8.9	1.7	181.8	7.9	10.1	1.9	7.8	2	10.5	3.5	18.8	7.3
6	11.2	0.4	197.8	0.6	10.5	0.9	7.9	0.4	10	0.5	14.1	0.7
6	13.7	0.7	194.8	1.8	15.2	0.9	10.5	1.5	13.5	2.3	18.3	3.5
6	13.1	0.5	193.1	1.7	15.3	0.3	9.4	0.4	11.5	0.4	15.5	0.6
7	11.4	0.3	192.3	1.6	11.8	0.3	8.1	0.3	10.1	0.4	12.6	0.6
7	12.2	0.7	196.9	6.1	13.1	0.7	9.4	0.7	11.9	0.8	16.8	1
7	12.5	0.2	192.1	0.4	13.9	0.7	9	0.1	11.1	0.5	15.6	0.2
average	12.2		190.2		12.8		8.7		11.0		15.7	
std dev	1.3		7.2		2.0		1.2		1.6		2.9	

Table A-6: Adult Human Blood Summary

Male

	Methylene chloride		Methyl ethyl ketone		Chloroform		Benzene		Trichloroethylene		Perchloroethylene	
age (yr)	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
23	14.9	0.1	178	0.3	15.8	0.5	9.4	0.5	12.1	0.6	15.1	0.7
26	14.8	0.4	181.9	2	16.6	0.6	10	0.6	13.1	0.6	17.3	0.6
36	12.5	0.2	185.3	1.7	12.3	0.1	9.2	0.6	12	1.8	17.7	7.1
39	13.1	0.5	181.4	3.2	16.6	0.7	10.6	0.7	14	0.8	20.4	1
39	13.2	0.4	186.1	2.9	16.4	0.3	9.8	0.2	13	0.2	16.7	0.2
39	12.6	0	182.4	1.6	15.4	0.2	10.3	0.1	13.1	0	20	0.2
39	14.4	0.8	179.7	3.1	16.4	0.6	10.6	0.5	14.1	0.6	19.8	0.9
41	11.2	0.3	191.1	2.7	12.8	0.3	8.3	0.3	11.7	0.4	16.4	0.7
47	13.8	0.6	186.1	1	14.9	0.4	9.5	0.1	12.4	0.5	17.4	0.3
48	9.9	0.4	168	0.8	9.7	0.1	5.5	0.3	6.7	1	6.7	2
48	13.2	0.2	181.6	1.4	11.6	0.1	7.6	0.4	9.2	0.9	10.8	1.2
48	12.4	0	189	7.2	13	0.8	9.5	1.7	12.2	2.1	16.9	4.8
48	13.4	0.3	191.6	2.2	11.9	0.2	8.1	0.2	10.5	0.1	13.5	0
49	13.5	0.8	192.4	0.4	12.7	0.6	8.3	0.4	11.4	0.6	13.9	0.3
51	11.5	1.9	182.8	0.9	11.2	2.3	7.2	3.1	7.8	4.6	7.6	6
51	12	0.1	179	1.3	14.3	0.3	8	0.1	10.7	0.1	14.2	0
54	11.4	0.4	186	1.7	12.1	0.3	8	0.3	10.6	0.1	13	1.5
54	11.3	0	194.1	1.2	12.4	0	8.7	0	11.6	0	16.6	0
55	13.8	0.5	180.3	1.7	16.8	0.5	10.8	0.5	14.5	0.4	19.9	0.4
57	12	0.1	171.8	2.2	13.7	0.4	8.7	0	10.9	0.1	15.1	0.1
58	13.5	0.1	180.7	2.1	13.4	0.2	8.8	0.1	11	0.1	14.8	0.1
61	13.1	0	176.7	2.9	13.7	0.4	8.9	0.1	11.4	0.1	14.6	0.2
61	12.7	0.7	189.4	0.5	12.6	0.7	8.7	0.6	11.5	0.6	16.6	0.1
62	17.1	3.2	205.2	32.8	18.1	3.7	12	3.5	16.8	5.5	21.9	8
64	14	0.4	174.2	5.1	14.2	0.8	8.9	0.5	11.5	0.4	14.5	0.2
66	13.1	0.3	183	2.7	15.3	0.5	10.3	0.5	12.9	0.6	17.5	0.5
68	12.4	0.1	195	0.6	11.5	0.2	8	0.2	10.4	0.1	13.3	0.1
68	13	0	194.8	3	12.5	0.1	8.8	0	11.6	0.1	16.5	0.1
70	12.1	0.4	180.1	0.3	14.2	0.3	9.6	0.3	12.5	0.3	18.9	0.4
72	14.1	0.1	184.3	3.4	13.6	0.3	8.9	0.3	11.5	0.2	15.8	0.1
80	15.7	1.1	197.5	3.8	11.7	1.1	8.6	1.1	10.8	1.2	16.6	1.3
82	11.4	0.6	179.2	0.3	13.3	0.6	8.3	0.1	10.8	0.1	14.5	0.4
average	13.0		184.6		13.8		9.0		11.7		15.8	
std dev	1.4		7.9		2.0		1.2		1.9		3.3	

Table A-6: Adult Human Blood Summary (continued)

Female

	Methylene chloride		Methyl ethyl ketone		Chloroform		Benzene		Trichloroethylene		Perchloroethylene	
age (yr)	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
19	11.5	0.1	192.2	2.4	11.6	0.2	8	0.3	10	0.4	14.5	1
20	14.5	0.5	185.4	3.2	13.8	0.4	9.4	0.3	12.5	0.3	18.8	0.2
21	12.7	0	184.2	2	14.3	0	9.2	0.1	11.2	0	15.8	0.3
22	13.4	0.7	191	6.3	11.1	0.2	8.2	0	10.6	0.1	17.3	0.6
25	11.7	0.1	188.7	0.3	13.1	0.2	8.6	0.2	11	0.2	15.1	0.3
27	12.1	0.5	196.9	0.6	11.7	0.5	8.9	0.6	11.2	0.5	18.3	0.5
30	12	0.2	191	1.1	12.3	0.3	8.6	0.4	11.1	0.4	16.8	1.1
35	10.7	0.7	170.3	5.8	12.8	0.8	7.2	0.8	9.6	0.7	15.2	0.6
38	13	0.3	177.7	3.1	13.4	0.2	7.7	0.1	10.1	0.1	14.8	0.2
38	11	0.1	193.4	0.7	12	0	8.2	0.1	10.6	0.1	15.2	0.2
41	12.7	0.4	184.8	0.2	13.8	0.3	8	0.3	10.3	0.2	14.8	0.2
43	16.9	0.6	185	0.4	16.4	1	10.6	1.8	14.4	3.1	20.9	5.1
44	7.9	1.1	179.9	0.5	7	1.7	4.2	2.5	4.8	4.3	7.9	10.2
44	11.7	0.6	189.1	4.4	12.6	0.6	8.6	0	11	0.5	14.5	0.9
45	13.3	0.2	188.3	2.1	13.9	0.1	8.8	0.1	11.2	0.1	16	0.1
48	12.8	0.3	197.5	1	11.5	0.3	8.3	0.1	10.8	0.3	17.3	0
50	14.6	0.1	187.9	2.5	14.6	0.1	9	0.2	11.3	0.1	15	0
55	11.8	0.2	195.2	1.2	10.7	0	8.2	0.3	8.7	0.7	13.4	1.2
55	13.5	0.7	189.9	7.9	12.8	0.2	8.9	0.4	11.8	0	16.7	2.1
57	12.2	0.1	177.5	2.6	13.5	0	7.5	0	9.6	0.1	12.7	0.3
58	12.2	1	190.2	3.7	11.5	0.6	8.3	0.6	10.8	0.8	16	0.4
63	13.3	0	181.3	1.6	13.3	0.1	10.4	0.4	13.2	1.2	19.5	3.4
68	11.7	0.7	192.5	2	12.2	0.3	8.5	0.2	11	0.1	15.8	1.4
71	13	0.3	191.6	6.8	11.3	0.5	7.7	0.5	9.3	0.4	12.3	0.8
80	14.2	1	184	3.2	15.1	1.2	9.9	1.4	12.4	1.7	15.3	1.9
82	15.6	0.3	185.4	0.3	15.1	0.6	10.8	1	13.7	1.3	20.7	1.7
87	7.7	0.3	176.1	0	6.9	0.2	2.9	0.1	3	0.1	2.3	0.1
average	12.5		186.9		12.5		8.3		10.6		15.3	
std dev	1.9		6.6		2.1		1.7		2.3		3.7	

APPENDIX B: FROZEN vs. FRESH DATA

To validate the use of frozen tissue for future work, partition coefficients were measured for each test compound in fresh and frozen tissues from four adult female rats. Half of each tissue was used immediately for PC determination (see Methods section). The remaining tissues were stored at -80°C for one week prior to thawing and PC determination. Compiled data from fresh versus frozen comparisons are in Tables B-1 through B-4.

Table B-1. Fresh and Frozen Tissue Comparison of PCs for Male Rat 1

			Methylene Chloride	MEK	chloroform	benzene	TCE	PCE
blood	fresh	mean	14.4	205.1	16.7	13.6	16.4	20.8
		s.d.	2.1	2.1	2.6	1.7	2.8	1.6
	frozen	mean	21.4	209.2	24.7	17.5	22.7	21.1
		s.d.	0.3	2.2	0.2	0.1	0.3	0.8
	% diff		48.4	2.0	47.9	28.7	37.9	1.4
liver	fresh	mean	24.8	186.0	21.5	20.6	26.1	48.8
		s.d.	1.3	7.2	0.5	0.6	1.4	4.9
	frozen	mean	21.4	168.2	23.1	22.3	29.1	56.5
		s.d.	0.3	4.9	0.7	0.6	0.7	2.1
	% diff		-13.7	-9.6	7.4	8.5	11.5	15.7
kidney	fresh	mean	14.2	230.6	16.8	15.5	19.3	37.0
		s.d.	2.1	17.0	1.4	1.2	1.2	1.1
	frozen	mean	12.3	178.1	17.3	16.3	20.8	43.3
		s.d.	0.3	0.4	0.4	0.3	0.3	0.9
	% diff		-13.2	-22.7	2.9	5.0	7.8	17.1
fat	fresh	mean	160.3	231.1	443.6	503.3	773.7	2036.5
		s.d.	2.4	0.6	3.4	1.8	2.2	1.2
	frozen	mean	154.7	240.0	425.4	481.9	739.8	1953.9
		s.d.	2.9	3.6	5.6	4.0	4.2	6.8
	% diff		-3.5	3.9	-4.1	-4.3	-4.4	-4.1
muscle	fresh	mean	10.2	171.6	14.0	11.9	15.3	33.7
		s.d.	0.4	0.8	1.1	1.1	2.0	4.0
	frozen	mean	11.4	162.9	17.0	15.1	19.5	39.6
		s.d.	0.4	2.8	0.7	0.9	1.2	2.8
	% diff		11.6	-5.0	21.1	26.5	27.2	17.7
brain	fresh	mean	12.2	173.0	17.1	18.8	24.6	58.8
	frozen	mean	11.6	173.4	16.4	17.3	22.1	51.3
	% diff		-4.7	0.3	-4.2	-7.9	-10.5	-12.9

Table B-2. Fresh and Frozen Tissue Comparison of PCs for Male Rat 2

			Methylene Chloride	MEK	chloroform	benzene	TCE	PCE
blood	fresh	mean	21.9	200.4	24.0	17.7	22.5	23.1
		s.d.	0.5	4.6	0.6	0.5	1.1	1.3
	frozen	mean	20.0	203.5	22.8	16.3	20.5	19.9
		s.d.	0.6	2.3	0.7	0.5	0.9	1.0
	% diff		-8.6	1.5	-4.8	-8.0	-8.6	-14.0
liver	fresh	mean	20.3	207.7	23.3	22.0	27.3	49.8
		s.d.	0.3	25.0	0.3	0.5	1.5	4.6
	frozen	mean	19.1	188.7	23.7	23.1	29.2	54.6
		s.d.	1.1	3.9	1.6	2.3	5.1	12.3
	% diff		-5.9	-9.2	1.5	5.1	6.9	9.5
kidney	fresh	mean	12.8	242.0	16.3	14.4	17.6	32.7
		s.d.	0.3	0.2	0.2	0.2	0.3	1.2
	frozen	mean	12.2	179.1	18.4	17.5	22.7	47.1
		s.d.	0.0	0.2	0.2	0.2	0.1	1.5
	% diff		-4.4	-26.0	12.9	21.8	29.1	44.0
fat	fresh	mean	159.5	230.3	444.7	500.1	770.1	2035.4
		s.d.	4.9	2.7	16.4	19.3	31.2	86.2
	frozen	mean	158.2	245.2	438.8	496.1	763.2	2019.0
		s.d.	1.8	4.4	2.9	4.6	8.2	24.3
	% diff		-0.9	6.5	-1.3	-0.8	-0.9	-0.8
muscle	fresh	mean	10.7	160.2	15.8	13.8	17.9	37.4
		s.d.	1.0	1.2	1.9	2.2	3.2	6.2
	frozen	mean	12.0	163.4	18.4	16.6	21.3	41.2
		s.d.	0.9	2.2	3.1	3.4	4.6	6.1
	% diff		11.6	2.0	16.8	20.4	18.9	10.4
brain	fresh	mean	11.5	171.4	16.6	17.5	22.4	50.5
	frozen	mean	13.2	175.5	19.3	20.6	26.2	55.7
	% diff		14.1	2.3	15.9	17.7	16.9	10.1

Table B-3. Fresh and Frozen Tissue Comparison of PCs for Male Rat 3

			Methylene Chloride	MEK	chloroform	benzene	TCE	PCE
blood	fresh	mean	22.2	199.2	24.0	16.9	22.3	20.8
		s.d.	0.7	3.4	1.0	0.7	1.2	0.7
	frozen	mean	21.7	202.1	24.1	17.1	21.6	20.3
		s.d.	1.0	3.8	1.2	0.8	1.2	0.9
	% diff		-2.0	1.5	0.4	0.8	-3.1	-2.0
liver	fresh	mean	20.0	335.9	21.0	18.6	21.1	35.3
		s.d.	0.5	59.3	0.9	1.0	1.6	3.9
	frozen	mean	16.7	177.0	21.1	20.8	26.3	48.4
		s.d.	0.8	8.4	1.0	1.0	1.8	3.3
	% diff		-16.2	-47.3	0.5	11.7	24.5	36.8
kidney	fresh	mean	14.2	210.8	21.1	19.3	23.4	41.6
		s.d.	0.3	4.1	0.5	0.3	0.6	2.1
	frozen	mean	12.8	170.4	19.6	19.1	25.4	54.9
		s.d.	0.3	3.7	0.7	0.7	0.9	4.3
	% diff		-9.6	-19.2	-6.9	-1.0	9.0	32.1
fat	fresh	mean	145.0	208.6	399.0	445.6	674.3	1738.1
		s.d.	8.9	19.8	27.8	31.8	49.6	129.1
	frozen	mean	151.6	232.3	416.4	472.4	725.9	8576.5
		s.d.	11.4	16.0	31.2	34.2	52.2	11596.3
	% diff		4.6	11.4	4.4	6.0	7.7	393.4
muscle	fresh	mean	10.5	159.8	14.9	12.4	15.2	30.3
		s.d.	0.6	1.7	1.3	1.5	2.1	4.8
	frozen	mean	10.8	162.7	15.9	13.9	17.8	34.4
		s.d.	1.0	2.7	2.9	3.2	4.7	8.1
	% diff		2.6	1.8	6.7	12.1	17.5	13.6
brain	fresh	mean	11.2	165.9	15.5	16.0	19.9	47.8
	frozen	mean	12.4	172.3	17.0	18.2	23.2	54.3
	% diff		10.8	3.9	9.8	13.8	16.8	13.6

Table B-4. Fresh and Frozen Tissue Comparison of PCs for Male Rat 4

			Methylene Chloride	MEK	chloroform	benzene	TCE	PCE
blood	fresh	mean	22.7	205.2	24.9	18.9	25.4	26.4
		s.d.	0.3	1.4	1.1	0.7	1.8	1.2
	frozen	mean	20.3	199.8	21.0	14.8	17.7	15.8
		s.d.	1.2	3.5	1.6	2.0	2.4	2.6
	% diff		-10.6	-2.6	-15.6	-21.7	-30.2	-40.3
liver	fresh	mean	23.6	207.5	22.5	21.8	28.6	57.7
		s.d.	0.9	29.5	0.6	0.5	0.7	6.7
	frozen	mean	17.4	162.5	21.0	20.2	25.0	45.8
		s.d.	0.6	0.8	0.9	1.2	3.1	7.1
	% diff		-26.5	-21.7	-6.8	-7.1	-12.4	-20.5
kidney	fresh	mean	13.9	195.4	19.6	18.8	25.1	51.4
		s.d.	0.2	1.8	0.4	0.4	0.4	1.4
	frozen	mean	13.0	173.9	19.8	18.8	24.3	49.8
		s.d.	0.6	5.4	0.4	0.6	0.4	5.7
	% diff		-6.2	-11.0	1.0	0.2	-3.1	-3.2
fat	fresh	mean	152.4	228.3	420.8	478.5	737.3	1949.4
		s.d.	6.1	4.5	16.9	21.2	33.2	90.7
	frozen	mean	157.9	243.9	432.7	489.0	740.4	1902.1
		s.d.	1.9	3.2	5.9	7.2	10.7	30.3
	% diff		3.6	6.9	2.8	2.2	0.4	-2.4
muscle	fresh	mean	10.4	160.2	15.1	13.1	16.9	36.5
		s.d.	0.5	2.0	0.8	0.8	0.9	3.2
	frozen	mean	9.9	158.1	14.6	12.4	15.6	30.4
		s.d.	0.7	2.0	1.8	2.0	2.8	4.7
	% diff		-4.4	-1.3	-3.0	-5.8	-7.9	-16.7
brain	fresh	mean	11.7	179.7	16.0	17.0	21.7	44.7
	frozen	mean	12.1	170.0	16.7	17.6	21.8	50.7
	% diff		3.6	-5.4	4.3	3.5	0.5	13.5

APPENDIX C: ANTICOAGULANT DATA

To verify that differences in anticoagulant used for human and rat blood did not cause variation in measured PCs, blood:air PCs were determined in rat blood treated with either sodium heparin or EDTA. Blood was collected from ten adult male rats and transferred to EDTA- or heparin-treated vacutainer tubes. PCs were determined (see Methods section) for all test compounds, and the results compared in Table C-1.

Table C-1. Effect of EDTA vs. Heparin on adult male rat blood:air PCs

		Methylene Chloride	MEK	chloroform	benzene	TCE	PCE
EDTA		22.7	196.3	24.5	17.6	22.4	20.7
		22.8	199.3	24.9	18.3	25.1	23.6
		23.3	199.9	25.4	19.4	27.4	27.5
		22.1	200.6	24.6	18.6	27.3	28.1
		23.0	203.4	25.7	18.9	29.1	28.7
	mean	22.8	199.9	25.0	18.6	26.3	25.7
	s.d.	0.5	2.5	0.5	0.7	2.6	3.4
Heparin							
		22.1	194.5	23.9	17.2	22.2	20.8
		22.7	198.3	25.2	18.4	25.2	23.8
		21.6	196.1	24.3	17.6	25.0	23.9
		21.8	200.1	24.9	17.9	26.8	25.9
		22.1	202.6	25.3	18.7	28.0	28.8
	mean	22.1	198.3	24.7	18.0	25.4	24.6
	s.d.	0.4	3.2	0.6	0.6	2.2	2.9
	% diff	3.1	0.8	1.2	3.3	3.2	4.5